**Original Research Article**

**Biological false positive rapid plasma reagin tests in pregnant females in North India**

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**Received:** 10 November 2020  
**Revised:** 04 January 2021  
**Accepted:** 15 January 2021

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**ABSTRACT**

**Background:** Syphilis, a sexually transmitted disease seriously complicate pregnancy and produce harmful results in fetus. Screening of pregnant females in early pregnancy with non-treponemal tests such as RPR and VDRL is a cost effective strategy for diagnosis of syphilis. However, these tests could produce BFP reaction in pregnancy. The prevalence of which may be different in different populations and at different times. The current prevalence of the biological false positive rapid plasma reagin tests in pregnant females of North India was there for assessed and being reported.

**Methods:** A total 500 consecutive pregnant females who presented to our tertiary care center for the first time were screened for syphilis by RPR (a non-treponemal test) and Hi-Quik (a treponemal test) after obtaining their written consent.

**Results:** Of the 500 females 21 (4.2%) were RPR positive and only one (0.2%) was Hi-Quik positive. Thus the prevalence of biological false positive rapid plasma reagin tests in pregnant females of North India was 4% (20/500). In semi quantitative RPR, these tests were positive in ≤1:4 dilution and were not found to be related to any particular age, geographical distribution, literacy status, occupation or period of gestation (p value >0.05) and were transient in nature.

**Conclusions:** Our study concludes that although RPR is a good, cost effective test for mass screening of pregnant females, but it produces BFP reactions in considerable percentage (4%) in pregnant females of North India.

**Keywords:** Antenatal screening, BFP reactions, Hi-Quik test, Pregnant females, RPR test, Syphilis

**INTRODUCTION**

Syphilis, a common sexually transmitted disease, with an estimated worldwide prevalence of 12 million new infections each year. It can seriously complicate pregnancy and may result in spontaneous abortion, stillbirth, non-immune hydrops feta lis, intrauterine growth restriction, perinatal death and serious sequelae in live born infectious children. It is still a challenge to diagnose this complex infection because of the inability of the culture of its causative agent, that is *T. pallidum* spp. *pallidum*. Antenatal screening of pregnant females provides a good opportunity to detect this infection in early stage of pregnancy. Historically the assays for non-specific antibodies have been used as routine screening tests for diagnosis of syphilis. This is because of their low cost and technical simplicity. However, the antigen utilized in these test is cardiolipin, which is non-specific and thus may cross-react with the sera of the patients who may not have syphilis.

Biological false positive reaction is defined as positive result in non treponemal test and negative result in treponemal test in the absence of syphilis and is not caused by any technical fault. The false positivity rates have been reported to be <1% in general population and
may be more in certain patient groups such as elderly or
the pregnant females or the patients with drug addiction,
malignancy, autoimmune disease (SLE), viral diseases
(Epstein Barr virus, hepatitis, HIV), protazol and
mycoplasma infection. Lubinski suggested in his study
that the percentage of false positive finding varies widely
even for the same disease by different authors. In
literature these reactions have been described as transient
and chronic. Transient are due acute processes and last
for less than six months. While chronic reactions persists
beyond six months and have been found in patients with
autoimmune diseases (rheumatic disorder) and
malignancies. It has also been reported that BFP reactions
comprise a higher proportion of reactions in a low syphilis prevalence population in comparison to high
syphilis proportion population.

Although, the exact prevalence of syphilis in India is not
known, but rising trends of syphilis from North India
have been observed in some studies. Therefore, the
present study was undertaken to assess the current
prevalence of BFP reactions in RPR test in pregnant
females, where this test is most commonly employed for
screening of syphilis in pregnancy.

METHODS

This cross-sectional hospital based study was conducted
on a total of 500 consecutively pregnant females who
visited the antenatal clinic of a tertiary care hospital of
North India from 3rd March 2019 to 3rd December 2019.

Inclusion criteria

All the pregnant females who were above the age of 18
years and were attending the antenatal clinic for the first
time were included

Exclusion criteria

Females <18 years and who were not resident of North
India were excluded from the study.

After taking their informed written consent their
demographic profile was recorded in the prescribed
proforma. Their blood samples were collected (using all
the sterile precautions). Serum was separated and stored
at 4°C. Rapid plasma regain (RPR), a slide flocculation
test was performed on the quantitative basis on the serum
samples using Recon Diagnostic Kit RPR card tests. Hi-
Quik immuno chromatographic test (ICA) was employed
to confirm the RPR positive test results. The Hi-Quik is a
rapid ICA for the qualitative detection of antibodies (IgG
and IgM) to T. pallidum in serum or plasma. In this test
recombinant syphilis antigen is immobilize in the test line
region of the test. After specimen is added to the
specimen well of the test cassette, it reacts with the
syphilis antigen coated particles and this mixture then
migrates chromatographically along the length and
interact with the immobilize syphilis antigen. The
positive result is indicated by a colored line in the test line
region and negative result by the appearance of color only
in the control line region and no colored line in the test
line region.

The study was approved by the Institutional Ethical
Committee. The data pertaining to the sociodemographic
profile was compiled and analyzed by using SPSS V.
20.0.

RESULTS

The study of sociodemographic profile of 500 females
showed that their age was between 18–40 years and mean
age was ±25.4. The maximum number of females
(40.8%) belonged to age group of 21-25 years, followed
by 32.2% in the age group of 26-30 years. Only 3.6%
females were more than the age of 35 years. Eighty four
percent female of the study group belonged to rural area
while only 16% were from urban area. Although most of
them (92%) were literate (had primary school education
or above), only 25.2% were employed. It was observed
that as many as 79% came to our tertiary care hospital
only in third trimester of pregnancy (Table 1).

Of these 500 females, 21 (4.2%) were found to be RPR
positive. Out of these 21 only 1 (0.2%) was Hi-Quik
positive and the test of 20 (4%) were Hi-Quik negative
(Table 1). This shows that biological false positive
reactions were present in 20 (4%) pregnant females of our
study. It was further observed that BFP reactions occurred
in dilutions of 1:4 or below on semi quantitative RPR test.
The maximum 9 (45%) false positivity was noted in 1:2
dilutions (Table 2).

The study of the 20 females who showed the biological
false positive reactions revealed that 35% of these false
positive reactions were shown by females each in the age
groups of 21-25 and 31-35 years. This was followed by
20% in age group of 26-30 years. However the difference
between these age groups were statistically not significant
(Table 1, p value: a and b=0.43; b and c=0.087). Out of
20 females showing false positive RPR, 17 (85%)
belonged to rural area and 3 (15%) were from urban area,
but the difference was statistically insignificant (p value
=1). Similarly the differences between the literacy status
(p value=0.75) and occupation (p value =0.79) of these
females with BFP reactions were also statistically
insignificant (Table 1).
Table 1: Sociodemographic profile and results of RPR and Hi-Quik tests of pregnant females (n=500).

<table>
<thead>
<tr>
<th>Sociodemographic characteristics</th>
<th>Total</th>
<th>RPR positive</th>
<th>Hi-Quik positive</th>
<th>False positive RPR</th>
<th>p value &lt;0.05 significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>47 (9.4)</td>
<td>2 (9.53)</td>
<td>-</td>
<td>2 (10)</td>
<td>a and c= 0.43</td>
</tr>
<tr>
<td>21-25</td>
<td>204 (40.8)</td>
<td>7 (33.34)</td>
<td>-</td>
<td>7 (35)</td>
<td>b and c= 0.87</td>
</tr>
<tr>
<td>26-30</td>
<td>161 (32.2)</td>
<td>5 (23.8)</td>
<td>1</td>
<td>4 (20)</td>
<td></td>
</tr>
<tr>
<td>31-35</td>
<td>70 (14)</td>
<td>7 (33.34)</td>
<td>-</td>
<td>7 (35)</td>
<td></td>
</tr>
<tr>
<td>36-40</td>
<td>18 (3.6)</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Geographical distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban &lt;sup&gt;e&lt;/sup&gt;</td>
<td>80 (16)</td>
<td>3 (14.3)</td>
<td>-</td>
<td>3 (15)</td>
<td>d and e=1.</td>
</tr>
<tr>
<td>Rural &lt;sup&gt;e&lt;/sup&gt;</td>
<td>420 (84)</td>
<td>18 (85.7)</td>
<td>1</td>
<td>17 (85)</td>
<td></td>
</tr>
<tr>
<td>Literacy status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate &lt;sup&gt;f&lt;/sup&gt;</td>
<td>43 (8)</td>
<td>1 (4.7)</td>
<td>-</td>
<td>1 (5)</td>
<td>f and g=0.75</td>
</tr>
<tr>
<td>Literate &lt;sup&gt;g&lt;/sup&gt;</td>
<td>457 (92)</td>
<td>20 (95.3)</td>
<td>1</td>
<td>19 (95)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed &lt;sup&gt;d&lt;/sup&gt;</td>
<td>375 (74.8)</td>
<td>17 (80.9)</td>
<td>1</td>
<td>16 (18)</td>
<td>h and i=0.79</td>
</tr>
<tr>
<td>Employed &lt;sup&gt;d&lt;/sup&gt;</td>
<td>126 (25.2)</td>
<td>4 ((19.1)</td>
<td>-</td>
<td>4 (20)</td>
<td></td>
</tr>
<tr>
<td>Period of gestation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; trimester &lt;sup&gt;j&lt;/sup&gt;</td>
<td>25 (5)</td>
<td>1 (4.7)</td>
<td>-</td>
<td>1 (5)</td>
<td>j and k=1.</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; trimester &lt;sup&gt;j&lt;/sup&gt;</td>
<td>80 (45)</td>
<td>1 (4.7)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; trimester &lt;sup&gt;k&lt;/sup&gt;</td>
<td>395 (79)</td>
<td>19 (90.6)</td>
<td>-</td>
<td>19 (95)</td>
<td></td>
</tr>
</tbody>
</table>

Figure in parenthesis represent percentage.

Table 2: RPR titres of RPR positive RPR and Hi-Quik positive test (n=21).

<table>
<thead>
<tr>
<th>RPR titre</th>
<th>RPR positive</th>
<th>Hi-Quik positive</th>
<th>False positive RPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>8 (38)</td>
<td>-</td>
<td>8 (40)</td>
</tr>
<tr>
<td>R2</td>
<td>9 (42.9)</td>
<td>-</td>
<td>9 (45)</td>
</tr>
<tr>
<td>R4</td>
<td>3 (14.3)</td>
<td>-</td>
<td>3 (15)</td>
</tr>
<tr>
<td>R16</td>
<td>1 (4.8)</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure in parenthesis represent percentage.

Figure 1: Seroconversion among BFP (n=20).

Of the 20 females who showed BFP RPR tests, affirmative tests for serology for all positive females were performed every week. On follow-up, the expected range for seroconversion was between 1 to 3 weeks. The maximum 10 (50%) number of females (with RPR reactive titre R1 and R2) completely returned to non-reactive state within 2 weeks followed by 3 (15%) (RPR titre R4) within 3 weeks (Figure 1).

DISCUSSION

Timely diagnosis and proper management of the syphilis in the pregnant females are important to prevent adverse outcomes of pregnancy. Primarily, screening with a non-treponemal test such as VDRL or the RPR is cost effective and is usually employed in antenatal clinics in India. Pregnancy is well recognized cause of biological false positive (BFP) reactions in non-treponemal tests. However, the percentages of reported false positive findings vary widely in literature. We studied the occurrence of BFP reactions in 500 consecutive pregnant females of our tertiary care hospital and the prevalence of BFP reaction was found to be 4%. This is much less than that observed in studies of Shaikh et al (13.88%) and Yassa et al (39%). This could be because their study populations included all suspected syphilis patients and not only pregnant females. In a similar study from North India BFP reactions were found in 0.27%, while it was 6.93% in the pregnant females of Nigeria.

Out of 20 females who show BFP reactions in our study, 9 (45%) had titre R2 and 8 (40%) had titre R1 (Table 2). The studies conducted by Taiwo et al and Smikle et al had also reported maximum BFP reactions in females having lower titres in RPR test. Out of 20 RPR reactive pregnant females, 7 (35%) each were from age group 21-
25 and 31-35 years. No female above the age 35 years were found to be reactive. The difference in the distribution of RPR reactivity between females of 31-35 years and 15-20 was statistically insignificant (p value 0.43). This shows that no specific age group is prone to show BFP reactions. Similar results have been reported by Mehta et al.17 The other factors studied for BFP reactions were geographical profile, education, occupation and all of these were found to have no statistical significant effect of BFP reaction (Table 1). In 2013 Mehta et al studied their effect of duration of pregnancy (trimester) and it was found to be statistical insignificant. This is in accordance with the result of the present study.17

In the present study the biological false positive RPR reactive sera underwent seroconversion after 1 week in 50% females, 2 week in 35% females and 3 week in 15% females (Figure 1). This indicates that BFP reactions were transient in nature. However all BFP reactors should be followed up and categorised as acute (transient) or chronic. Patients with chronic BFP results should be investigated for clinical laboratory indicators of autoimmune or other chronic diseases. These measures would reduce the indiscriminate and inappropriate use of antibiotics and other medicines. Wiwanitkit studied 30 patients who had biological false positive VDRL test and on follow up the seroconversion range was between 9.25 and 10.49 weeks.19

Unfortunately, our study had limitations of small sample size collected from the pregnant females visiting the single health care facility only.

CONCLUSION

The results of the present study emphasize the need to continue the use of cost effective RPR tests for screening of syphilis of pregnant females to prevent its adverse effect of pregnancy. However, the females showing positive RPR test should be carefully followed up by either doing confirmatory test or by retesting (RPR test) to rule out BFP rapid plasma reagin tests.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee BFUHS/2K19P-TH/8904

REFERENCES